wherein at least one of the substituted amino acids is aspartic acid-12, tyrosine-15, tyrosine-17, histidine-35, or asparagine-38; and

wherein the mutant is substantially nonlethal compared with a protein substantially corresponding to wild type SPE-C toxin.

REMARKS

Applicants have received and reviewed the Office Action dated September 26, 2000. By way of response, Applicants have cancelled claims 2 and 11-16 without prejudice, amended claims 1, 3-5, 7, and 9, and added new claims 17-19. Claims 1, 3-10, and 17-19 are pending. No new matter is introduced. Applicants submit that the amended and newly presented claims are supported by the specification.

In particular, support for the amendment to claim 1 reciting an amino acid substitution in a β-barrel of a B-subunit or a N-terminal alpha helix can be found in the specification at least at page 12, line 8 to page 14, line 21.

For the reasons given below, Applicants respectfully submit the amended and newly presented claims are in condition for allowance, and notification to that effect is earnestly solicited.

Petition for Extension of Time

A two-month extension of time is necessary to provide the response. A request is made for an extension of time from December 26, 2000 to February 26, 2001 for responding to the office action.

Restriction Requirement

Applicants acknowledge that the Examiner has made the restriction requirement. Applicants have cancelled claims 11-16 to the non-elected invention.

Specification

Applicants have checked the specification and noted several typographical errors, which are corrected by the amendment to the specification hereinabove.

35 USC § 112, First Paragraph Rejections

The Examiner rejected claims 1 - 10 based on 35 USC § 112, first paragraph alleging insufficient support for every possible insertion, deletion or substitution of one or more amino acids. Applicants respectfully traverse this rejection.

Applicants note that the amended and newly presented claims relate to mutants of SPE-C including particular substitutions at particular amino acids or in particular structural domains. Each of these residues and structural domains are specifically called out in the present specification as preferred locations for substitutions. For example, the specification supports the recitation in claim 1 of a mutant comprising an amino acid substitution in a β-barrel of a B-subunit or a N-terminal alpha helix. At least page 12, lines 8 - 17 of the specification supports mutations on β-barrel 4 of B-subunit 5. Particular amino acids supported as points for mutation in the β-barrels include His-35, Asn-38, Thr-33 and Leu-36. At least page 14, lines 14 - 22 of the specification supports mutations on N-terminal alpha helix 51. Particular amino acids supported as points for mutation in the N-terminal alpha helix include Ser-11, Asp-12, Tyr-15 and Tyr-17. Further, Example 6 (pages 39 - 43) provides support for a number of single and double mutants of SPE-C: Y17A, N38A, Y17S, N38S, N38A, Y15A/N38A, Y17A/N38A Y15S/N38S, and Y17S/N38S. This description of suitable regions of the protein provides disclosure commensurate with the scope of the claim for producing nonlethal mutations. The claims recite mutations in these secondary structural features.

The Examiner further alleges that there is no substantive evidence that the claimed vaccines are capable of inducing protective immunity. Applicants respectfully disagree. The double mutants Y15A/N38A, Y17A/N38A, Y15S/N38S, and Y17S/N38S were prepared in Example 5 and tested for capacity to enhance endotoxin shock in Example 6. After giving a group of rabbits either a mutant SPE-C toxin or SPE-C wild type toxin, the animals were challenged with *Salmonella typhimurium* endotoxin. The rabbits that had been given mutant SPE-C toxin survived whereas those that had received the wild-type toxin died (see Table 4 on page 40).

Further studies involved immunization of rabbit groups with 2 weekly doses of SPE-C double mutants. Blood samples before and after immunization were compared for antibodies against purified streptococcal derived wild type SPE-C. The blood samples after immunization had higher levels of antibodies (see Table 5 on page 41).

The immunized animals were then challenged with wild type SPE-C and then 4 hours later with *Salmonella typhimurium* endotoxin. None of the rabbits that were immunized died whereas rabbits that were not immunized died (see Table 6 on page 41).

By describing the discrete residues and specific structural features of the protein that are suitable for making mutations that yield a nonlethal SPE-C as well as showing specific examples of mutations that caused immunization against wild type SPE C and endotoxin, Applicants have met the standard for enablement under § 112, first paragraph.

The Examiner alleges that the specification does not provide guidance on how multiple amino acids can be deleted, substituted or inserted for the production of a stable protein. The Applicants respectfully disagree with the Examiner regarding the relevancy of protein stability for this invention. An important requirement for a mutant to function as a vaccine is nonlethality rather than stability. The protein does not have to remain intact to function as a vaccine. There is support for claims that the mutants can be used as vaccines. Four double mutants (Y15A/N38A, Y17A/N38A, Y15S/N38S, and Y17S/N38S) were prepared as described in Example 5 and then evaluated in Example 6. The mutations were effective as vaccines. Finally, there are no claims in the present invention regarding the stability of the mutations. Therefore, it is believed that questioning the stability of the mutant is inappropriate.

The Examiner cites several references dating between 1984 and 1991 to support assertions regarding protein stability. Applicants respectfully submit that the state of the art for determining and analyzing protein structures between 1984 and 1991 is not relevant to enablement of the present application. The present application claims priority to a filing date in December 1996. Enablement of the present application should be judged based on the state of the art regarding mutant proteins as of this December 1996 filing date. The protein arts advanced considerably between 1991 and December 1996, and especially between 1984 and December 1996. For example, it was only after 1991 that x-ray crystallography became a routine method for determining protein structures, and modeling studies of three-dimensional structures of proteins also advanced in that time period. Therefore, Applicants respectfully submit that it is inappropriate to depend on teachings of these old articles and books to support an enablement rejection. Applicants respectfully request the Examiner withdraw the comments regarding these references and the rejection based on these references.

Applicants respectfully submit that the claims are fully enabled by the specification and request withdrawal of this rejection.

35 USC § 112, Second Paragraph Rejections

The Examiner rejected claims 1-10 under 35 U.S.C. § 112, second paragraph. The Examiner objected to certain terms and phrases employed in the claims. Applicants respectfully traverse this rejection.

The Examiner suggested spelling out the words forming the acronym SPE-C the first time its used in a claim. Claim 1 has been amended as suggested by the Examiner.

The Examiner objects to the recitation in claim 1 of the phrase "substantially nonlethal". Applicants respectfully direct the Examiner's attention to the specification as filed at page 18, lines 9 to page 19, line 7. This passage describes that the claimed mutant SPE-C toxins are substantially nonlethal in rabbits when administered by miniosomotic pump (as described in Example 4) at the same or greater dose than a wild type SPE-C toxin. Specifically, the mutant SPE-C is substantially nonlethal if when administered to a rabbit at the same dose as the wild type toxin, less than about 10-20% of rabbits die. Applicants respectfully submit that the phrase "substantially nonlethal" is clearly defined by the specification.

The Examiner noted inadvertent typographical errors in the numbering of certain amino acid residues in the specification including Table 2. Applicants have corrected these errors by the present amendment to the specification. Applicants note that the specification clearly states that numbering of amino acid residues is made by reference to the sequence of Figure 1 (specification at page 9, lines 25 - 27). The amendments to the specification bring the text into accord with the sequence numbering in Figure 1.

The Examiner objected to the recitation in claim 10 of the phrase "substantially enhanced endotoxin shock". Applicants respectfully direct the Examiner's attention to the specification at least at page 20, lines 13 - 20. This passage describes that a lack of enhancement of endotoxin shock can be evaluated in rabbits as described in Example 3. Substantially no enhancement of endotoxin shock is seen when less than about 25% of the animals develop shock when the mutant SPE-C toxin is co-administered with endotoxin as compared to wild type SPE-C activity at the same dose. Preferably, substantially no enhancement of endotoxin shock results in no

animals developing shock. Therefore, Applicants respectfully submit that the phrase "substantially enhance endotoxin shock" is well defined in the specification as filed.

Accordingly, it is believed that the claims fully comply with § 112, second paragraph, and withdrawal of this rejection is respectfully requested.

Rejection of Claims Under 35 USC § 102

The Examiner rejected claim 1 under 35 U.S.C. § 102(b) as anticipated by *Goshorn et al.* (Infection and Immunity, 56 (9): 2518-2520 (1988)). Applicants respectfully traverse this rejection.

The standard for anticipation requires that a single document disclose every element of a claim. The *Goshorn et al.* reference fails to disclose any mutants of SPE-C, much less any substantially nonlethal mutants of SPE-C. This reference merely discloses the amino acid sequence of the wild type SPE-C. Therefore, the *Goshorn et al.* reference cannot anticipate the presently claimed invention.

The Examiner cites the *Goshorn et al.* reference for its description of "deletion subclones" employed to clone and sequence the wild type SPE-C (*Goshorn et al.* at page 2518, paragraph bridging columns 1 and 2). Applicants are uncertain how the creation of deletion subclones while cloning and sequencing a gene for wild type SPE-C relates to the present claims. Applicants respectfully submit that deletion subcloning refers to a procedure in which the ends of a large piece of DNA are trimmed away to provide a smaller piece of DNA with an intact coding sequence. Each of these deletion subclones included an intact SPE-C wild type coding sequence. The attached Declaration Under 37 C.F.R. § 1.132 by Dr. Schlievert, one of the present inventors and one of the authors of the *Goshorn et al.* reference, states that this was the type of deletion subcloning conducted. Further, deletion subcloning does not produce any protein. Therefore, this procedure does not produce a protein that might fall under claim 1 of the present application.

The Examiner asserts that the *Goshorn et al.* reference suggests using site directed mutagensis to analyze the SPE-C toxin. Applicants respectfully note that the *Goshorn* reference states "Future studies will utilize site-specific mutagenesis to analyze such regions." (*Goshorn et al.*, page 2519, column 2, last sentence of first full paragraph). That is, the *Goshorn et al.* reference states that it would be desirable to continue studies employing a technique commonly used for characterizing proteins. No such mutant was made, and no structure of any such mutant

is described. Therefore, this recitation does not teach any SPE-C mutant or a sequence encoding that mutant. The Examiner's logic in employing this passage would require that any mutant of any coding sequence or protein would be obvious based on knowledge of the wild type sequence and the existence of the technique of site directed mutagenesis. Such logic is not in accord with the patent law.

The Examiner then goes on to describe that the *Goshorn et al.* reference discusses similarities between the SPE-C amino acid sequence and amino acid sequences of other toxins. *Goshorn et al.*, however, fail to mention even a single hypothetical mutant of SPE-C. Therefore, this discussion fails to teach any aspect of the present claims.

The *Goshorn et al.* reference cited by the Examiner describes the sequence of a wild type SPE-C protein and the DNA encoding it. This reference suggests that it might be desirable to study this protein through site-specific mutagenesis. The reference fails to mention even a hypothetical mutant of either the SPE-C protein or DNA encoding such a mutant. Therefore, this reference cannot anticipate any claim of the present application.

Accordingly, based on the foregoing differences, it is respectfully submitted that the reference applied by the Examiner neither teaches nor suggests the presently claimed invention, and withdrawal of this rejection is respectfully requested.

Rejection of Claims Under 35 USC § 103 - Goshorn et al. in view of Kline et al.

The Examiner rejected claims 1 - 10 under 35 U.S.C. § 103(a) as obvious over *Goshorn et al.*, in view of *Kline et al.* (Infection and Immunity, 64(3): 861-869 (1996)) Applicants respectfully traverse this rejection.

As discussed above for the anticipation rejection, the *Goshorn et al.* reference neither teaches nor suggests the presently claimed mutant SPE-C. The *Kline et al.* reference does not remedy the shortcomings of the *Goshorn et al.* reference.

First, the *Kline et al.* reference is not properly considered as prior art against the present application. The *Kline* reference was published in March of 1996, which is less than one year before the priority date of the present application, December 6, 1996. The attached Declaration Under 37 C.F.R. § 1.131 by Dr. Patrick Schlievert, filed in the parent case, states that the present invention was developed before the publication date of the *Kline et al.* reference. Therefore, the *Kline et al.* reference is not properly considered as prior art against the present application.

Further, even if it were prior art against the present application, the *Kline* reference does not remedy the shortcomings of the *Goshorn et al.* The *Kline et al.* reference discloses residues of the SPE-A toxin that are said to be important to T-cell mitogenicity and for class II MHC binding. It is believed that portions of the SPE-C protein relevant to these biological activities are not necessarily relevant to lethality (present specification at paragraph bridging pages 18-19). The present application describes complex and detailed modeling studies done with the SPE-C molecule to determine secondary structural features and residues that are important for producing a nonlethal SPE-C mutant. There is no suggestion or even mention in the *Kline* reference of how a skilled worker might accomplish such modeling to discover such structural features and residues, or of any other way to develop a nonlethal SPE-C mutant. Therefore, the *Kline et al.* does not remedy the deficiencies of the *Goshorn et al.* reference, and does not provide any teaching or suggestion of the presently claimed invention.

Accordingly, the combined references cited by the Examiner neither teach nor suggest the present mutants and withdrawal of this rejection is respectfully requested.

Rejection of Claims Under 35 USC § 103 - Goshorn et al. in view of Hartwig et al.

The Examiner rejected claims 1 - 10 under 35 USC § 103(a) as obvious over *Goshorn et. al.* in view of *Hartwig et. al.* (International Immunology, 5(8): 869-875 (1993)). Applicants respectfully traverse this rejection.

As noted above, *Goshorn et al.* does not disclose, nor does it discuss, the secondary structure of the mutant SPE-C toxin recited in claim 1. Rather, *Goshorn et al.* teaches only the primary structure of the SPE-C toxin by disclosing the amino acid sequence for SPE-C. There is no teaching of any particular mutation that would be substantially nonlethal compared with a protein substantially corresponding to wild type SPE-C toxin.

Hartwig et al. does not remove the deficiencies of *Goshorn et al. Hartwig et al.* discloses several mutations of SPE-A and the effect of these mutations on T lymphocyte stimulatory activity. Replacements of several amino acids of SPE-A with alanine were carried out by site-specific mutagenesis. There are some similarities between the structures of SPE-A and SPE-C but they are different proteins and *Hartwig* discloses nothing about SPE-C. None of the mutations reported by *Hartwig et al.* for SPE-A correspond to the residues recited in the present claims. The residues disclosed by *Hartwig* are about 40 or more amino acids away and on a

different part of a different protein. *Hartwig et al.* only discloses mutations to SPE-A and does not suggest any mutations to a different protein such as SPE-C, particularly to a portion of the protein that does not correspond to where the mutations were made in SPE-A. There is no suggestion that non-lethal mutations of SPE-C can be prepared by substituting an amino acid in a ß-barrel of a B-subunit or a N-terminal alpha helix. There is no suggestion that one type of secondary structure is preferable for making nonlethal mutations of SPE-C. *Hartwig et al.* does not suggest specific mutations corresponding to aspartic acid-12, tyrosine-15, tyrosine-17, histidine-35, or asparigine-38 in SPE-C. Rather, *Hartwig* suggests mutations at a different part of a different protein.

The combination of references does not disclose or suggest the important characteristic of non-lethality. Rather, the combination of *Goshorn et al.* and *Hartwig et al.* only suggests that mutations can be made and that some mutations might affect T lymphocyte activity. However, disclosing how to produce a mutation does not teach how to make a mutation that is non-lethal. The combination of references does not suggest that altering amino acids in a ß-barrel of a B-subunit or a N-terminal alpha helix can produce non-lethal mutations of SPE-C. Further, the combination of references does not suggest the specific residues claimed in the present invention.

Accordingly, it is respectfully submitted that the combination of *Goshorn et al.* and *Hartwig et al.* does not make the present invention obvious. Withdrawal of this rejection is respectfully requested.

Summary

Claims 1, 3 - 10 and 17 - 19 are in condition for allowance. The Examiner is invited to contact Applicants' undersigned representative at the telephone number listed below if doing so will expedite the prosecution of this patent application.

Respectfully submitted,

Schlievert et al.
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